Expression of flagella

- Preculture
- Inoculate 6x40 mL and 1x1L expression culture with 1:1000 preculture
- Incubate culture for 16 h at 33°C, 200 rpm
- Take SDS sample
- Harvest cells at 2500 g, 4°C for 10 min

Isolation of flagella

Chemicall isolation of flagella

- Cell pellet of 1 L culture is resuspended in 60 mL motility buffer (10 mM potrassium phosphate buffer, 67 mM NaCl, 0.1 mM EDTA) by centrifugation at 1600 g for 30 min at 4°C
- The rinsed solution is centrifuged at 2500 g for 20 min and resuspended in 8 mL of saline solution (67 mM Nacl, 0.1 mM EDTA)
- Adjust the pH of the solution to about 2 with the addition of 1 M HCl
- Stirr the bacteria constantly for 30 min by vortexing at a moderate setting while maintaining the pH at about 2. Pellet deflagellated cell bodies by centrifuging for 1 h at 105000 g and 4°C at pH 2
- Collect the resulting supernatant containing the flagella monomers

Mechanical isolation of flagella

- Resuspend cell pellet of 40 mL cultures in 1 mL Phosphate buffer each
- Shear cells with a syringe (0.8 mm diameter) thirty times by fill and release of cell suspension
- Centrifuge sheared suspension at 14000 rpm for 15 min (Eppendorf 5418 R)
- Collect supernatant and store at -20°C to 4°C

Making flagella seeds

- For generating assembled flagella filaments in vitro flagella polymerization seeds are required: These are formed by taking a small portion of the flagella monomers (i.e. isolated chemically) is added to an equal volume of 2 M Na2SO4, 10 mM Potassium phosphate buffer pH 6.5 to create polymerization seeds
- Polymerisation solution was incubated for 1 h
- The solution is centrifuged at 105000 g for 1 h and resuspended in 150 mM KCl, 10 mM potassium phosphate buffer pH 6.5

The solutions of flagellin monomers and the seeds are combined and allowed to polymerize into long flagellas filaments for 24 h